DIGITAL X-RAY CAMERA FOR QUALITY EVALUATION THREE-DIMENSIONAL TOPOGRAPHIC RECONSTRUCTION OF SINGLE CRYSTALS

Introduction

Background of the Invention

X-ray topography is a well known method for
10   characterizing the microstructure of single crystals. It
uses x-ray diffraction to measure defects and imperfections
in crystal structures. A defect in crystalline structure
affects how well the protein structure can be determined.
Many of the basic components of organisms, e.g. proteins,
15   DNA, RNA, can be crystallized. A defect in protein
structure may affect the function of that protein, possibly
resulting in disease.

There are currently many x-ray topography devices and
methods of identifying defects in crystals using white beam
20   or monochromatic x-ray topographs. The white beam technique
(Laue geometry) provides rapid imaging but results in low
effective resolution. Protein crystals tend to suffer
severe and rapid degradation from the intensity of a white
synchrotron beam. However, this technique is useful for
25   rapidly screening samples. Monochromatic beam methods can
provide more detailed information but require careful
alignment and longer exposures. (Stojanoff et al., **1996**,
Acta Cryst., A52, 498-499) (Stojanoff et al., **1997**, Acta
Cryst., D53, 588-595) (Vetter et al., **2002**, Acta Cryst.,

D58, 579-584) (Boggon et al., **2000**, Acta Cryst., D56, 868-880).

U.S. Patent 6,385,289 (Kikuchi) teaches a device using two-dimensional information of x-ray intensities and 5 a charge coupled device (CCD) camera with larger pixels (12 or 24μm) than the present invention (less than 10μm, preferably 8μm) as described for x-ray topography. The device measures x-ray rocking curves using a movable x-ray detector which measures x-ray intensities diffracted off of 10 the surface of a sample. Kikuchi's method diffracts x-rays off of a surface and measures the angle of the diffraction and the intensity of the x-rays when they strike the surface of an x-ray detector. In contrast, the present invention uses transmission geometry, where the x-rays are 15 diffracted by passing through a sample.

U.S. Patent 6,468,346 (Arnowitz et al.) teaches a method of improving crystal growth using magnetic fields or varying levels of gravity. Both earth and space grown crystal samples were studied. The space grown crystals were 20 found to exhibit higher crystallographic perfection. X-ray topographic images are used to reveal defects in the crystals and to permit identification of the sets of conditions that produce crystals having the fewest defects. A method to generate topographic images is not taught.

25 Japanese patent 2000314708 teaches an x-ray topography apparatus with a movable stand, x-ray source and irradiation side slits which regulate x-rays from the x-ray source toward a specimen. The radiation side split is arranged between the specimen and a CCD sensor that detects 30 x-rays from the specimen. A specimen support frame holds the specimen while it is measured.

U.S. Patent 6,498,829 (Borgstahl et al.) teaches a method for mosaic spread analysis which uses super fine ϕ-slicing data collection, unfocused monochromatic radiation 35 and a suitable fast readable area detector such as a CCD.

Random radiation events in the phosphor or optical taper
intensities are removed from the diffraction data to create
reflection profiles.

5   Most CCD systems presently used for crystallography
have a fiber optic bundle in front of the CCD relative to
the x-rays. The fiber optic bundle is generally tapered
like a cone with the small end at the CCD. This provides a
detecting area larger than the CCD chip. A phosphor is
required because the fiber optic bundle is unable to
10 transmit x-rays. The phosphor converts the x-rays into
visible light. The fiber optic bundle also helps to protect
the CCD from the damaging x-rays by absorbing the rays that
are not converted by the phosphor into visible light. The
fiber optic bundle has the disadvantage of reducing the
15 spatial resolution of the CCD.

There is a need for a topography system that is low
cost, rapid, quantitative and directly reads out to
electronic media. Traditionally, single crystal topography
was employed using x-ray film or nuclear emulsion plates.
20 The currently available technology has some disadvantages,
including long exposure times, slow sampling rate and
chemical processes that can vary according to the protocol
used, and the age of the reagents used. The present
invention overcomes these problems by using a digital
25 camera, giving the user real time imaging, minimizing
exposure times, and eliminating the variable chemical
processes used by conventional methods.

Summary of the Invention

30   The present invention provides a digital topography
imaging system comprising an x-ray emitting source which
comprises a shutter; a sample holder; a charge coupled
device (CCD) camera which measures reflection profiles from
the x-ray emitting source; a means for controlling pixel
35 image corruption due to pixel overloading in the CCD

camera; and a means for acquiring and displaying images of a sample.

The digital topography imaging system of the present invention may be employed to determine crystalline

5 structure of a sample.

Description of the Figure

Figure 1 shows the system assembly of the present invention.

Detailed Description of the Invention

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Topography is the high spatial resolution imaging of the individual parts of a crystal contributing to an X-ray reflection. (Otálora et al., J. Cryst. Growth, **1999**, 196, 546-558). Topography provides insight into the internal

15 diffraction properties of a crystal (Stojanoff et al., Acta Cryst., **1996**, A52, 498-499)(Stojanoff et al., Synchotron Radiation News, **1996**,9, 25-26)(Stojanoff et al., Acta Cryst., **1997**, D53, 588-595)(Dobrianov et al., Acta Cryst., **1998**, D54, 922-937)(Otálora et al., J. Cryst. Growth, **1999**,

20 196, 546-558)(Boggon et al., Acta Cryst., **2000**, D54, 868-880). The lattice deformations surrounding defects within the crystal produce the contrast in the topographic image. By quantifying these data, one can compare the quality of different crystals.

25   Typically, images are acquired with a fine-grain x-ray sensitive film or nuclear emulsion plates. The fine grain size (typically 2-3μm with film and down to 1μm with nuclear emulsion) has been the sole reason film and nuclear emulsion plates have been utilized for so long in X-ray

30 topography. Drawbacks to using film include slow processing time, variation between batches of film and processing chemicals, and a limited dynamic range. Repeatable, high

accuracy, quantitative analysis with film-based techniques
is difficult.

These problems arise because the film must be digitized
before a computer can process the data. As part of the

5   digitization process, the frames must be geometrically
corrected and registered with one another. Even with
fiducial marks, this process can be time consuming. Film-
based processes are slowly being replaced with digital
equivalents in many fields.

10   The present invention uses a CCD to capture digital
topography images directly. Some benefits of using a CCD
are that it is extremely fast, can be very sensitive, and
the data are automatically digitized and registered.
However, the spatial resolution is normally not as high as

15   film, and dark current limits the exposure time of the
system. This problem can be minimized by lowering the
operating temperature of the CCD.

There are tradeoffs in the design of a CCD chip. For
example, if the pixel size is reduced to increase the

20   spatial resolution, the electron well capacity is reduced,
which also reduces the dynamic range.

For use in topography, spatial resolution is the most
important parameter. Therefore, a CCD with small pixel
size (8μm) was selected for the present invention. In a

25   previous study, a CCD-based x-ray camera was used to study
topography of synthetic diamonds. (Ludwig et al., J. Appl.
Cryst., **2001**, 34, 602-607) However, Ludwig et al., used the
CCD with a powder phosphor to convert the x-rays to visible
light and a fiber-optic faceplate to channel the visible

30   light to the chip surface for detection.

The present invention uses the relatively weak
diffraction of macromolecular crystals and direct detection
of x-rays by the CCD chip. The CCD chip used in the present
invention incorporates anti-blooming circuitry. With anti-

35   blooming circuits, spatial resolution is preserved and

intensity information is lost in pixels which overflow. The preservation of spatial resolution is very useful for topography.

5   The present invention comprises a low cost, real time digital topography imaging system for evaluation of three-dimensional topographic reconstruction of single crystals, which may replace x-ray film and nuclear emulsion plates. The present invention uses a CCD camera with a very fine pixel size (8μm) to convert x-rays into an electrical

10   signal.

The imaging system directly converts x-rays to an image without the use of phosphor. The CCD camera of the imaging system is designed for short exposure applications of less than one second and therefore has a relatively high dark

15   current leading to noisy, raw images. The resulting noisy, raw images are then processed to sharpen them, providing a clearer image of the data.

The digital topography imaging system of the present invention comprises an x-ray emitting source; a sample

20   holder; a charge coupled device (CCD) camera with antiblooming circuitry which reduces pixel image corruption due to CCD camera pixel overloading; positioning systems for the camera and the sample holder; and a means for acquiring and displaying images that have been processed

25   for clarity.

The x-ray emitting source further comprises a shutter, allowing for variable exposure times. The x-ray emitting source must provide x-rays that are nearly parallel and have a bandwidth of less than 1%. The x-ray beam must be

30   large enough to cover all of the sample, or the region of the sample that is of interest. Exposure time is dependent on the strength of the source and the nature of the sample. The method of the present invention can be used with exposure times of any length.

The sample holder can be any suitable size or material. The sample holder is preferably moveable so that the sample can be positioned in the line of the x-ray beam. The holder must not translate or allow the sample to vibrate during

5 data collection. The sample holder is rotated. Movements of the sample holder may be computer controlled. In the present invention, a positioning system controls the CCD camera. The camera positioning system may also be computer controlled.

10 The CCD chips used in most crystallographic applications are expensive, so the protection offered by the fiber optic bundle is necessary. The CCD chip used in the present invention is inexpensive, when compared to the chips used in other applications. The CCD of the present invention

15 does not require protection from x-ray damage. The intensity of the x-rays striking the surface of the CCD, in the present invention, is reduced because the x-ray beams are diffracted by the sample prior hitting the CCD.

The charge coupled device (CCD) camera measures

20 reflection profiles from the x-ray emitting source. The CCD camera has a very fine pixel size of 10 μm or smaller. An 8 μm pixel size was used in one embodiment, however, smaller pixel sizes are preferred. Using the CCD camera with antiblooming circuitry allows for accurate measurement of a

25 very bright pixel next to a very dim pixel and the high spatial resolution provides a detailed image of the reflection.

Antiblooming circuitry is used as a means for controlling pixel image corruption due to CCD pixel

30 overloading (blooming). Blooming occurs in a CCD when the charge accumulating in a pixel spills over into neighboring pixels. The result is a strong signal in one pixel, appearing as a large spot. Such a large spot may occupy many pixels and drown out weaker signals in the neighboring

35 pixels. Antiblooming circuitry provides sharp detail

between regions of the crystal, even when neighboring pixels are overloaded.

5 After an exposure, an image of the sample is captured from the CCD camera directly to memory in a computer. The time it required for the data from the CCD to transfer to computer memory is known as "read out time" and is typically much less than a second. As each pixel is transferred from the CCD to memory, the electrical charge accumulated in the CCD for each individual pixel is 10 converted to an integer value. The actual range of values is dependent on the resolution of the analog to digital converter (ADC). This resolution is measured in bits with a typical ADC having a resolution between 8 and 16 bits. The ADC is part of the computer interface card that connects 15 the CCD camera to a computer system and is commercially available.

A computer program assigns a color to each value for each pixel. Pixels with the same value receive the same color as defined in the color map. A color image of the 20 sample is displayed on a screen. The color image represents the intensity measured for each pixel. The image may be scaled to fit the screen.

In one embodiment, a method for digital topography imaging comprises placing a sample in the sample holder of 25 the digital topography system of the present invention, positioning the sample holder between the x-ray emitting source and the CCD camera, exposing the sample to x-rays from the x-ray emitting source, measuring the x-ray reflection angles of the x-rays that are passed through the 30 sample from the x-ray emitting source with the CCD camera to form a raw image, and processing the raw image measured by the CCD camera with a computer program which provides clearer images of the structure of the sample, and displaying those images on a screen.

   In another embodiment, the raw image produced using the
above method is processed using a multi step method to
remove the background and system noise. The first step is
to remove the dark current noise by subtracting a dark
5 current image of equal exposure. Any electric ripple noise
is removed using a wavelet transform. The wavelet transform
comprises four filters: a low frequency decomposition
filter, a high frequency decomposition filter, a low
frequency reconstruction filter, and a high frequency
10 reconstruction filter. Then, a histogram cut-off filter is
used to reduce the remaining background noise. A median
filter is used to clean up the speckled nature of the data.
A wavelet transform is used to enhance resolution. A
histogram filter is used to filter out any remaining low
15 level noise. The data is then expanded and digitized into
an integer range of 0-255. A color table having assigned
colors for each numerical value is created with the integer
value to determine how effective the processing eliminated
noise from the data. For example, a value of zero is white.
20 Values of 1-255 progress incrementally from blue to red.
Results having an integer value close to zero in the
background region are desired.

   After processing, animations are constructed with the
corresponding reflection profile to provide the diffraction
25 of the crystal volume versus the oscillation angle, as well
as composite images depicting parts of the crystal.

   The present invention preferably uses a two-dimensional
positioner to place the CCD camera and its holder into the
x-rays emitted from the source or diffracted from the
30 sample. The positioning system used in the present
invention may be created by connecting two Velmex BiSlides™
together at right angles to each other. Positioning
products from other vendors are also acceptable for use.

   As shown in Figure 2, in a preferred embodiment, the
35 system assembly of the present invention comprises a base

with an inlaid screw, placed on a horizontal flat surface.
The horizontal BiSlide **5** is mounted to the vertical BiSlide
4. The vertical screw **7** allows the horizontal BiSlide **5** to
move up and down the length of the vertical BiSlide **4**,

5   relative to the horizontal flat surface. The camera **1** is
mounted to the horizontal BiSlide **5** by the mounting bracket
2 and the camera mounting bracket **3**. The horizontal BiSlide
5 has an inlaid screw allowing the mounting bracket **2**,
camera mounting bracket **3**, and camera **1** assembly to move

10   laterally along the length of the horizontal BiSlide **5**.
Both the vertical and horizontal screw assemblies allow the
camera **1** to be positioned where needed when the topography
imaging system of the present invention is used.

    The topography imaging system of the present invention

15   may be mounted onto a software controlled, x-y-2Θ
positioning stage that can be positioned in front of a
QUANTUM4™ CCD detector for the collection of topographs. For
example, the QUANTUM4™ is used for measuring the intensity
of hundreds of reflections simultaneously. It measures the

20   integrated intensity of the reflections, but does not
provide data on the internal structure of the reflection.
In the present invention, the CCD detector is used to
locate diffracted beams in terms of x position, y position
and angular range from the crystal over a large area. The

25   CCD camera is then used to collect high spatial resolution
topography on the individual beams.

    Although topographs will only be measured in the
vertical orientation, the horizontal axis will be used to
center the reflection in the detector and to compensate for

30   spot movement as the reflection passes through Ewald's
sphere.

    The present invention is further illustrated in the
following, non-limiting examples.

Examples

Example 1.

The CCD topography system is based on an Electrim Corporation EDC-2000S camera which uses a Texas Instruments
5 TI-TC281 CCD chip. For this experiment, Texas Instruments provided a TI-TX281-31 CCD, which was a TC281 with the cover glass removed.

The TC281 has an 8x8 mm active area with consisting of a 1000x1000 array of 8µm square pixels. The pixel well
10 capacity is 32,000 electrons. The CCD contains special anti-blooming circuitry. EDC-2000S offers software-programmable adjustment of the gain, offset, and exposure time.

NSLS Beamline X26-C was used for this experiment. It was
15 configured for unfocused monochromatic radiation at 1.52 Å. The assignments of the motor axes differ from conventional Eulerian cradle geometries typically used.

In an effort to simulate a conventional setup the ω axis was held stationary so that the ψ axis was held
20 perpendicular to the x-ray beam. The axes φ and χ were used to position the reflections so that they were directly above the beam in order to minimize the effect of beam divergence and Lorentz broadening on the reflection profile (Bellamy et al., Acta Cryst., **2000**, D56, 986-995). The 2Θ
25 and ν motions were used to position the detectors. After a reflection was located with the scintillation counter, the topography camera was inserted in front of the scintillation counter and held in place with custom sized length of clear reusable single sided bonding strip. The
30 camera and the x-ray shutter were synchronized manually; the camera exposure was 10 seconds and the x-ray shutter time was 5 seconds.

The images were recorded using a software package. The images are written to disk in TIFF format (Aldus

Corporation 1986), which allows image-processing concepts to be prototyped and analyzed with MATLAB (The Math Works Inc. 1992).

The raw data images from the camera were quite noisy and
5 were passed through a seven step processing sequence to improve the images. The first step is to remove the dark current noise by subtracting a dark-current (no x-rays) image of equal exposure time. The TC-281 CCD was designed for use in short exposure time environments (<<1s) and has
10 a significant dark current when the exposure time is on the order of seconds, as it was in this case.

The second step is to remove an electrical ripple noise that is present over the entire surface of the CCD. The wavelet transform was used to reduce the ripple noise. The
15 wavelet transform provides several advantages over the Fourier transform for manipulating images (Mallat, "A Wavelet Tour of Signal Processing", 1998). The two most important are that all of the coefficients are real and that frequency information is localized. The wavelet
20 transform consists of four filters derived from a scaling function and a related wavelet function. These filters form orthonormal basis functions in the filtering space in much the same way that sine and cosine form basis functions in Fourier space. Decomposition low-pass filter (L), a
25 decomposition high pass filter (H), a reconstruction low pass filter and a reconstruction high pass filter were used. A wavelet coefficient was calculated by taking the dot product of the filter with a group of pixel values. The filter was indexed across the image two pixels at a time,
30 and a coefficient was calculated at each location (Strang and Nguyen, "Wavelets and Filter Banks", 1996). For a two-dimensional image, the filters are first applied along the horizontal direction and then along the vertical direction leading to a two-letter convention that describes the
35 filter process.

A channel labeled LH indicates that the L filter was applied along the horizontal followed by the H filter along the vertical. For an image at the first stage of decomposition there are four channels named LL, LH, HL and

5   HH. The decomposition channels each contain 1/4 the number of pixels in the original image so that the total number of pixels remains unchanged after the transform is applied.

When shown on an image, the upper left is normally LL, upper right is LH, lower left is HL and lower right is HH.

10 A channel can undergo a further decomposition and the resulting sub-channels follow the same convention. When the image was decomposed with a sixth order Daubechies wavelet (Daubechies, "Ten Lectures on Wavelets", 1992) most of the complex noise appeared in the coefficients of the LH

15 channel.

To reduce the complex noise, a one level decomposition was performed followed by a reconstruction with LH decomposition removed. The resulting image has a more evenly distributed background with lower overall intensity

20 and less structure.

For the third step, a histogram cut-off filter was used to remove the remaining background noise from the image and leave the intensity near the reflection. The histogram filter found the average and standard deviation of all

25 pixels and then set each pixel with a value less than the mean, plus 2.5 standard deviations to zero. The fourth step was to apply a median filter to clean up the speckled nature of the data. The median filter replaces the value of each pixel with the median value of a box of surrounding

30 pixels.

The fifth step was to use the wavelet transform to enhance the resolution to roughly 4x4μm. To enhance the resolution, the image from the fourth step was submitted to the inverse wavelet transform as the LL decomposition. The

35 other three channels were set to zero. The resulting

reconstruction has double the resolution. This approach is reasonable because in most wavelet decompositions the majority of the signal, in terms of total energy, is contained in the LL channel. The sixth step was to use a

5 histogram cut off filter and clean out any remaining low-level noise. The method of step 4 was used.

The seventh step was to expand and digitize the data back into the integer range 0 to 255, by examining the entire sequence and using the maximum and minimum values to

10 scale all the images in the sequence. The color table used in all of the reflection images has level zero as white and then levels 1 through 255 going from blue to red. Using white as level zero shows how well the processing was able to eliminate the noise from the data.

15 **Example 2.**

Sequences of topographic images were collected on two different reflections from a large cubic-insulin crystal (>500x500μm). The sequences were collected as a series of stills about the ψ axis.

20 Image sequences for Crystal A were taken. The image sequences from Crystal A reveal an area of the crystal that stayed in diffracting condition for a longer amount of time in all images. The second reflection from Crystal A appears to be composed of three large diffracting areas, two

25 smaller ones on the right side of the crystal, and a larger one on the left side. There is a small gap of almost no diffraction dividing those areas.

Comparison of the Crystal A image sequences for reflections 1 and 2 show that images are significantly

30 degraded between reflection 1 and reflection 2. Reflection 2 yields an image that is speckled and of a lower intensity. Possible reasons for this degradation include radiation damage, dehydration, and the possibility that this reflection would normally have a weaker intensity.

Example 3.

Sequences of topographic images were collected on four different reflections from a second large cubic-insulin crystal (>500x500 μm). The sequences were collected as a
5 series of stills about the ψ axis.

Crystal B was mounted and used exclusively for topography measurements. All measured reflections of Crystal B were well defined and the signal was significantly above the background. The post experimental
10 picture of Crystal B shows no yellowing of the crystal, indicating that it suffered less radiation damage than Crystal A.

The images of Crystal B's topography show it to be more complex than Crystal A. Crystal B had sharp transitions
15 between areas of the crystal that were diffracting during the specimen rotation, rather than the smooth transitions seen during the rotation of Crystal A.